Herbage Studies. I.—Lotus corniculatus, *a Cyanophoric Plant.*

By Henry E. Armstrong, F.R.S., E. Frankland Armstrong and Edward Horton.

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Hitherto little attention has been paid to the individual plants which constitute the herbage of pasture lands and no serious attempt has been made to appraise their quality; this is the more surprising, as it is well known that certain pastures are of special value as grazing lands and that the food value of herbage often differs to an extraordinary extent in different districts and even in neighbouring fields—so much so that it is impossible to fatten cattle on many, if not on the majority, of pastures; moreover, there are marked differences depending on seasonal conditions. It is clear that such differences may be due both to variation in the botanical composition of the herbage and to variation in the composition of individual plants induced by variation in soil and in climatic conditions; at present, however, it is impossible even to hazard an opinion as to the manner in which these and doubtless other factors are operative.

Our present difficulty arises from the lack of methods of appraising quality: we are no longer satisfied with determinations of dry matter, digestible matter and albuminoid nitrogen, now that we realise that *quality* as much as *quantity* is of importance—that in the case of cattle, as in our own case, a mixed and varied diet is required and that what may be termed the condimental constituents of food are, perhaps, at least equal in importance to those which serve exclusively as building materials or as a source of energy. An increasing weight of evidence appears to be in favour of the view that the vital processes in plants as well as in animals are controlled in greater or less degree by substances of the class we have proposed to designate collectively as Hormones. There can be little doubt, in fact, that it will be necessary to take many factors into account in appraising the value of foods—far more, indeed, than it has been customary to consider hitherto.

It is not at all improbable that the glucosides present in plants in small quantity are in some cases of definite *condimental* value. A case in point is that of linseed. Owing to the presence of the glucoside linamarin (phaseolunatin) in the unripe seed, a small quantity of hydrogen cyanide is usually to be found in linseed cake. It is well known that this cake has qualities which make it superior to all other seed cakes as a food in bringing cattle into condition; it may well be that it owes its superiority to this small amount of hydrogen cyanide and perhaps also to the acetone

that accompanies the cyanide. Arrangements have been made which it is hoped will permit of this problem being solved.

We have been fortunate in having our attention attracted to a plant the study of which promises to be of interest not only from the point of view above set forth but also for other reasons which will be apparent when our account is considered. In the course of our search for enzymes of the emulsin type* we have examined a large number of Leguminosæ and were led, early in the summer of last year, to discover in *Lotus corniculatus* (Bird's-foot trefoil) a plant in which such an enzyme is associated with a cyanophoric glucoside. We may mention that another reason which led us to select this plant and test it for hydrogen cyanide was the fact that Dunstan and Henry had discovered this substance in *Lotus arabicus*—a plant growing on the banks of the Nile—and that hydrogen cyanide had also been found in *Lotus australis*.

The first specimen tested was picked on the Thames, near Wargrave, in June, 1910. It was found to contain hydrogen cyanide when tested by Guignard's alkaline picrate paper: a slip of the yellow paper, enclosed in a small tube with two or three grammes of the plant and a drop or two of chloroform, soon darkened in colour and ultimately became brick-red. This specimen of *Lotus corniculatus* was also found to contain an enzyme or enzymes which acted readily both on linamarin and on prunasin† though but slightly on amygdalin.

Of several specimens obtained from the Reading district soon after the first was picked, only one or two contained hydrogen cyanide; moreover, the cyanide could not be detected in a number of specimens picked in July in the Harpenden district and also near Flitwick (Beds.).

During the early part of August search was made for the plant all over the Swanage district, in Dorsetshire. It was found growing on London clay, on chalk, on Purbeck and Portland limestone and on Kimmeridge clay but only in one or two cases was hydrogen cyanide detected; no difference was apparent between the plants from the various soils.

In the latter part of August we met with the plant in Switzerland, in the Saas Valley; again no evidence of the presence of hydrogen cyanide was obtainable.

In September and October we obtained a second set of specimens from the Harpenden and Flitwick districts; these also were tested without cyanide being discovered.

* Compare 'Roy. Soc. Proc.,' 1910, B, vol. 82, p. 349.

† We propose to use this name for the glucoside prepared from amygdalin—*amygdo-* or *mandelo-nitrile-glucoside*—sometimes spoken of by us in earlier communications as Fischer's glucoside.

One specially interesting result of the work done at this time may be mentioned here. The form of *Lotus corniculatus* which some botanists regard as a mere variety and others as a distinct species, *Lotus major* or *Lotus uliginosus,* which grows, as a rule, in damp situations, was found to be free not only from hydrogen cyanide but also from the correlated enzyme. This variety is distinguished by its rank growth and coarse tubular stem.

The conclusion we arrived at last year was, therefore, that *Lotus corniculatus* occasionally contained a cyanophoric glucoside and corresponding enzyme but we had no reason to connect the presence of the glucoside with any particular conditions either of soil or of climate.

This year the first specimen of Lotus we examined was sent to us from Portrush, in North-East Ireland, by Dr. J. Vargas Eyre, who early in May found a dwarf form of the plant growing there in profusion on the sand dunes. This proved to be rich in hydrogen cyanide and also contained an active enzyme. Dr. Eyre obtained other specimens in Ireland during May; all of these were cyanophoric.

At Whitsuntide, however, one of us tested a considerable number of specimens in Ayrshire, in the Barrhill district, always without finding any trace of cyanide; but on going out to the coast at Ballantrae again a stunted form of *Lotus corniculatus* was found growing in profusion on the beach just above high-water mark and this plant contained both cyanide and enzyme but other specimens obtained on the same day from the hillside overlooking the beach and only a short distance from it were free from cyanide.

Having found cyanide only in the two stunted forms of the plant grown on sea-sand at the coast, we were led to think that the occurrence of the cyanophoric glucoside might possibly be favoured by "starvation conditions," especially as the conditions during the previous year and in the Ayrshire district early in the present season had been such as to favour luxuriant growth.

During the present summer specimens have been procured from many localities; the result of testing these has been to show that whereas last year cyanide was rarely present, this year it has rarely been absent. We have never failed to detect it in plants from the neighbourhood of Reading, grown under all sorts of conditions, excepting always the form definitely recognisable as *Lotus corniculatus* var. *major* (*uliginosus*); wherever we have obtained this form, it has always proved to be free from cyanide and we have also confirmed our observation made last year that this variety is free from the enzyme which occurs in the cyanophoric form.

Plants growing this year under a great variety of manurial conditions on the experimental grass plots at Rothamsted have always contained cyanide;

last year we never succeeded in detecting it in plants growing on these plots. But it is very noteworthy that on several occasions this year we found patches of the plant in the Harpenden district growing near to one another which were markedly different, the one being rich in cyanide the other containing little if any. Thus of five separate patches found on July 1 in a field of lucerne, only three contained an appreciable amount of cyanide; of two patches growing close together at the edge of a wheat field only one contained cyanide; a case similar to this latter was met with at Redbourn, a few miles from Harpenden.

We had a like experience with plants from Yorkshire. Mr. Harold Wager was good enough to send us seven specimens collected early in July near Threshfield, in Yorkshire, at spots which appeared to afford somewhat different conditions; five of these were rich in cyanide, whilst two contained but traces.

Plants collected in various places in the Isle of Wight in August were all very rich in cyanide. It was also found in plants growing in the Swanage district in places where none could be detected in the specimens collected last year.

Plants have been raised by one of us, at Lewisham near London, from seed gathered last year at Kimmeridge from plants (growing on the cliff face in disintegrated Kimmeridge shale) which did not then contain cyanide. From an early stage onwards up to the present date (November 20, 1911), these have always contained cyanophoric glucoside and the attendant enzyme. We regard this as a result of special importance.

Plants have also been raised from seed obtained early in the year from Messrs. Vilmorin, of Paris, at Lewisham, at University College, Reading, and on four of the barley plots at Rothamsted—1A, 2A, 3A and 4A; these have always been rich in cyanide. Plants raised at Lewisham and Reading from seed purchased from Messrs. Vilmorin as that of *Lotus major* var. *villosus* have shown no trace of cyanide and have also been free from enzyme.

Plants obtained at West Horsham in July, at Margate in September and at half a dozen different localities in the Sidmouth (Devonshire) district, also in September, were all cyanophoric.

One other experience remains to be related with reference to the British Isles. Early in September, on visiting St. Andrews at the time of the celebration of the 500th Anniversary of the University, one of us found *Lotus corniculatus* growing in several places. A plant of somewhat rank growth occurring in grass of rank growth at the roadside near Largoward, Fife, did not afford cyanide but this was detected in a plant of less luxuriant growth found in the same locality in short grass bordering a carriage drive. An extraordinarily dwarf form of the plant was found growing on the sea

face of the sand dunes bordering the St. Andrews Golf Links; hydrogen cyanide was not detected in this specimen.

This and last year, it is well known, afford a most remarkable contrast, as in the two seasons the weather has been of very different and opposite types—wet, cold, dull weather having prevailed during the summer of last year (1910), whilst this year (1911) has been characterised by long-continued drought, accompanied by high temperatures and an altogether unusual amount of sunshine.

Our home experience would lead us to correlate the appearance in *Lotus corniculatus* of the cyanophoric glucoside and the attendant enzyme with conditions such as have prevailed during the present year—with conditions favouring maturity rather than luxuriance of growth. But apparently some allowance should be made for a factor of variability, which perhaps is Mendelian, on account of differences observed even during the present phenomenal summer in plants growing under conditions which appear to be very similar if not identical.

In this connection, the following account given of *Lotus corniculatus* in Bentham and Hooker's 'Handbook of the British Flora' is of interest:—

L. corniculatus, Linn., *Bird's-foot Trefoil.*—Stock perennial, with a long tap-root. Stems decumbent or ascending, from a few inches to near 2 feet long. Leaflets usually ovate or obovate; stipules broader than the others. Peduncles much longer than the leaves. Umbels of from five or six to twice that number of bright yellow flowers; the standard often red on the outside. Calyx-teeth about the length of the tube. Pod usually about an inch long. Seeds globular, separated by a pithy substance, which nearly fills the pod.

In meadows and pastures, whether wet or dry, open or shaded, widely spread over Europe, Russian and Central Asia, the East Indian Peninsula and Australia but not reaching the Arctic Circle. Abundant all over Britain. Flowers the whole summer. It is a very variable species, accommodating itself to very different stations and climates; and some of the races appear so permanent in certain localities as to have been generally admitted as species but in others they run so much into one another as to be absolutely indistinguishable.

The most distinct British forms are:—

(a) *L. uliginosus*, Schk.—Tall, ascending or nearly erect; glabrous or slightly hairy and luxuriant in all its parts, with six to eight flowers in the umbel. Calyx-teeth usually but not always finer and more spreading than in the smaller forms. In moist meadows, along ditches, under hedges and in rich, bushy places. *L. major*, Sm.; *L. pilosus*, Beeke.

(b) *L. crassifolius*, Pers.—Low and spreading, often tufted at the base, glabrous or nearly so, usually with five or six rather large flowers to the umbel. Leaflets broad and often glaucous, especially near the sea, where they become much thicker. In open pastures and on dry, sunny banks.

(c) *L. villosus*, Coss. and Germ.—Like the common variety but covered with long, spreading hairs. In dry, sunny situations, common in Southern Europe but in Britain found only in Kent and Devon.

(d) *L. tenuis*, Waldst and Kit.—Slender and more branched than the common form, with very narrow leaflets. In poor pastures and grassy places, chiefly in South-eastern Europe; rare in Britain, always running much into the common form. *L. decumbens*, Forst.

We have not had an opportunity of testing varieties *b* and *c* but have found what we believe to be the variety distinguished as *tenuis* in the Isle of Wight. This has proved to be particularly rich in cyanide.

At times we have thought that size of leaf and degree of hairiness were in some way correlated with the occurrence of cyanide but this has not proved to be the case. There is, however, very little doubt that, as a rule, the dwarf forms are richer in cyanide and that luxuriance of growth favours the disappearance of cyanide.

During August this year one of us has had the opportunity of testing the plant in many places in Norway in the Bergen and the Christiania districts. It was found growing in profusion on the Island of Holsenöe off Bergen, at Voss on the lake shore and on banks at the roadside, at Os in grass and on the roadside near Norheimsund. It was rampant on the moraines at the foot of the Boium and Suphelle glaciers at Fjaerland (Sogne-fjord) and on the Buer glacier at Odda (Hardanger-fjord). Specimens were also secured at Notodden, at Tinnoset and at Eidvos. Dr. Solberg of the Statens Kemiske Kontrolstation at Trondhjem was so kind as to send us a specimen picked at Charlottenlund near Trondhjem. In no single case could cyanide be detected in the Norwegian plant. Of four specimens tested for enzyme, only two contained an appreciable amount and neither came up to the average English plant in activity.

This result appears to us to be very remarkable, especially when the opinion is taken into account which prevails among botanists that both colour and odour are more highly developed in northern regions where light is active during a greater number of hours than it is in our British region.*

Having given most careful attention to the condition of vegetation generally in Norway during August this year, the opinion one of us formed was that the condition everywhere was distinctly and definitely one of relative immaturity and somewhat exuberant growth wherever the circumstances were such as to favour growth. This was particularly noticeable in red currants and raspberries. These fruits, it is well known, grow to a far larger size in Norway than here but they lack the character of English-grown fruit—they appear to be less acid, less sweet, less flavoured and far more "watery." The final impression left was that the conditions in Norway are

* The argument is also applied to Alpine plants (*cf.* R. R. C. Nevill, 'Journal of the Royal Horticultural Society,' October, 1911, vol. 37, p. 77).

such as to favour continued growth rather than ripening. From this point of view, it may be questioned whether the specially brilliant colour of Norwegian flowers—if it be a fact—may not be less a consequence of any direct action of light and more an outcome of the greater supply of the colouring agent conditioned by the longer continuation of growth under northern conditions, the supply not being cut down by the setting in of the ripening process at an early stage. Even in our own climate, flowers are apt to be very brilliant in colour in spring and early summer.

Fortunately we have been able to extend our observations this year practically over the whole of Europe. Dr. J. Vargas Eyre, who has been studying the growth of flax on behalf of the Development Commission, has been able to collect and test Lotus for us at a large number of localities. We are greatly indebted to him for the following summary of his observations:—

Date.	Place.	Situation.	Character of plant.	HCN.
	FRANCE.			
July 11	Dunkirk	Hot sand	Low growing..................................	Trace
November 15	,, ...	Amongst grass on sheltered bank	,, spreading habit..............	Distinct
	BELGIUM.			
July 16	Werelghem	Moderately moist	Moderately luxuriant	None
,, 17	Courtrai	Warm, damp (? *L. major*)	Very tall (over 2 feet), hollow stem, large leaves	None
,, 18	Werelghem	Sun scorched railway track	Very stunted..................................	None
	HOLLAND.			
,, 28	Hoorn	Moderately moist	Common type, moderately luxuriant......	None
,, 30	Enkhuisen	,, ...	,, large broad leaves	None
August 5 ...	Bolsward and Arum	(1) Sandy, sun scorched cinder track	Very dwarf type, no sign of flowering...	None
		(2) Wet ditch...........	Slender, branched, narrow leaves (? var. *tenuis*)	Yes
		(3)	Moderately luxuriant, found with (2) ...	None
,, 9 ...	Arum	Grass bank	Apparently same as (2)	Yes
	GERMANY.			
,, 24 ...	Potsdam	(1) Lawn of new palace	Dwarf habit, large flowers, very small leaves	None
		(2)	Common type	None
	RUSSIA.			
September 2	Alt-Schwanenburg	Moist	Common form, luxuriant	None
,, 29	Orel	(1) River bank, moderately damp	Dwarf habit, small narrow leaves, few in number, hollow stem	None
		(2) ,, ,,	Similar to (1) but large amount of foliage	Yes
		(3) ,, ,,	Similar to (1), except leaves were shorter	Yes

Date.	Place.	Situation.	Character of plant.	HCN.
	RUSSIA—*contd.*			
October 1 ...	Zmyarka (50 miles S. of Orel)	(1) Moderately moist	Somewhat erect habit, stalky, few narrow pointed leaves, hollow angular stem	Yes
		(2) " "	Creeping habit, much branched, many small blunt leaves, hollow round stem	Yes
		(3) " "	Sturdy, woody growth, very small flowers, long narrow drooping leaves	None
		(4) " "	Succulent plant, broad leaf, large blossom	Trace
		(5) " "	Succulent, abundant large broad leaves, creeping habit	None
" 2 ...	Orel	(1) Upland fields	Bushy, much foliage, small narrow leaves	Yes
		(2)	Large, stalky growth, few rather broad leaves	Yes
		(3)	Dwarf habit, very small pointed leaves, much foliage	Yes
		(4)	Large flowers, dwarf plant, very small pointed leaf	Yes
		(5)	Large growth, long leaf, large seed pods	Yes
		(6)	Dwarf habit, broad ended leaf, large flowers	Yes
" 4 ...	Moscow	(1) Damp garden	From E. Russian clover seed, very luxuriant, hollow stem, large long leaves (? *L. major*)	None
		(2) "	From American clover seed, less luxuriant than (1), full stem, smaller pointed leaves	Yes
		(3) "	From S. Russian clover seed, full stem, fewer blunt ended leaves, more dwarf habit	Trace
	AUSTRIA.			
" 11 ...	Trautenau	(1) Kapellenberg, 3000 feet	Small leaf, dwarf plant, much branched, full stem	Yes
		(2) " "	More luxuriant than (1) but dwarf, large leaves, full stem	Yes
		(3) " "	Small, creeping, narrow leaf	Yes
		(4) " "	" " small leaf, dark stems...	None
	HUNGARY.			
" 23 ...	Szeged	(1) Open field	Rather small leaves, thin stems, creeping, much branched plant, somewhat hairy, leaves glaucus	Yes
		(2) "	Leaves very narrow and sharp pointed, rather hairy plant, large stems, upright	None
	ITALY.			
November 2	Bologna	(1) Botanical garden...	Weak growth, angular stems, smooth plant, small narrow leaves (*L. tenuis*)	Very distinct
		(2) Three miles S. of Bologna	Small growth, smooth plant, small narrow leaves, luxuriant	Yes
		(3)	Similar to (2) but more luxuriant growth	Yes
		(4)	Similar to (3) but much more luxuriant, in warm, moist situation	Yes
" 6	Florence	(1) Hills about $1\frac{1}{2}$ miles from Florence	Fresh growth from old root, pointed leaves of moderate size	Faint
		(2) " "	Similar to (1) but leaf round ended ...	Distinct
		(3) " "	" " smaller growth.........	Faint
		(4) " "	" (2) but "	Distinct

It is probable that, in some cases, the plant Dr. Eyre examined was the *major* variety [Courtrai, Moscow (1)]. On the whole, his observations confirm those we have made at home and in Norway; those which he made in Moscow at the garden of the Agricultural Experiment Station may be referred to as specially interesting: in order to bring under the notice of students the manner in which weeds are introduced with agricultural seeds, neighbouring plots there had been sown with clover seed from various localities. *Lotus corniculatus* was among the weeds on each of the plots but specimens from three plots behaved quite differently: no cyanide was present in the plant—probably the *major* variety—growing on the plot sown with seed from East Russia but that from the plot sown with American seed was extraordinarily rich in cyanide, whilst that derived from seed from South Russia contained but little cyanide. The East Russian plant had a very large leaf; the American plant, rich in cyanide, had the smallest leaf. It may be mentioned that Dr. Eyre could not find *Lotus corniculatus* in the St. Petersburg district.

It is clear that *Lotus corniculatus* is a variable plant, though more often than not, during the present year, it has contained hydrogen cyanide everywhere except in Norway, where the climatic conditions are undoubtedly somewhat special.

Taking into account the variability of the plant and the occurrence of cyanophoric and acyanophoric plants in close proximity, there is reason to suspect crossing and it will be important to carry out experiments from this point of view. But it will be necessary to isolate the varieties first and study these apart. We have secured seed this year in Norway as well as from other places and hope that we shall be able to raise plants next year which will make it possible to state whether the non-occurrence of cyanide in the Norwegian plant is in any way a consequence of climatic influences.

As to the nature of the glucoside present in *Lotus corniculatus:* we have detected acetone as well as hydrogen cyanide and taking into account the fact that the Lotus enzyme acts so readily on linamarin, it is highly probable that the glucoside present is *linamarin*—the glucoside characteristic of many varieties of flax—and that the enzyme is the *linase* which is associated with linamarin in flax. In this connection, the following observations are of interest:—

		Percentage activity of enzyme towards			
		Linamarin.	Prunasin.	Amygdalin.	Salicin.
Lotus corniculatus...	July 10, 1910	64·5	32·0	2·7	27·8
„ *major*	August 10 „	1·8	2·0	1·5	—

Unfortunately, we have not yet been successful in our attempts to isolate the glucoside: either the amount present has been too small for us to separate it from the large amount of other substances which are extracted with it or we have been unfortunate in our choice of method.

The following typical determinations of hydrogen cyanide obtainable from the plant may be quoted as showing that the amount present is but small:—

Source and date (1911).		Per cent. HCN.
Portrush. May 22		0·055
Ballantrae. June 7		0·017
Rothamsted (Wild). June 10		0·019
Rothamsted (grown on the barley plots from French seed)	1A. July 24	0·049
	2A. ,,	0·044
	3A. ,,	0·037
	4A. ,,	0·030
Lewisham—Kimmeridge. September 28 (grown at Lewisham in 1911 from seed collected at Kimmeridge in 1910)		0·048

Taking the highest value (0·055 per cent.), at most about 0·5 per cent. of linamarin can have been contained in the plants examined by us. Unfortunately, in drying the plant, even when this is done very carefully and rapidly at a low temperature, much of the glucoside is destroyed. It will be desirable therefore to work with the undried plant in future.

To determine the enzymic activity of the plant we usually expose it to the action of toluene and afterwards dry it rapidly, *in vacuo*, over oil of vitriol. The dried material is then finely ground. To carry out the determination, 20 c.c. of an M/5 solution of linamarin (0·9886 grm.) is digested with 0·2 grm. of the leaf material during 24 hours at 37°. The amount of hydrogen cyanide liberated is then determined in the manner described in No. XIII of our "Studies on Enzyme Action."

The typical results given on p. 481 may be quoted in illustration.

From the experience gained during the past year, it is clear that in future it will be necessary, in all cases in which hydrogen cyanide is not detected, to test for enzyme as well, so as to discriminate between plants in which both cyanide and enzyme are present and those in which enzyme is present but no cyanide—and to ascertain whether these latter alone have temporarily lost the power of storing the cyanide.

	Source and date.	Per cent.
L. corniculatus	Thames Bank, July 18, 1910	64·5
L. major	Swanage, August, 1910	1·8
L. corniculatus	Portrush, May 22, 1911	94·5
"	Ballantrae, June 28, 1911	38·8
"	Rothamsted (Wild), June 10, 1911	92·5
"	Threshfield, Yorkshire, June 27—	
	Small leaves	82·5
	Large "	82·75
"	Lewisham (from Vilmorin's seed), July 13, 1911 ...	90·5
"	Courtrai (J.V.E.), July 17, 1911—	
	(? var. *L. major*), Hairy	1·5
	Non-hairy	1·0
"	Rothamsted, July 24, 1911—	
	From Vilmorin's seed: Field 1A	83·5
	" 2A	84·75
	" 3A	85·25
	" 4A	81·75
"	Margate	82·75
"	Lewisham (from Kimmeridge seed)	88·0
"	Fjaerland, August, 1911	48·5
"	Vik, August, 1911	17·3
"	Notodden, August, 1911	0·5
"	Trondhjem, October, 1911	0·6
	Vilmorin's seed	3·75
L. major	Cirencester, July 26, 1911	0·5
"	Lewisham (from Vilmorin's seed), July 29, 1911 ...	1·0
L. tenuis	Isle of Wight, August, 1911	79·75

It is well that we should put on record the results obtained on growing *Lotus corniculatus* at Rothamsted on four of the barley plots, 1A–4A, across each of which a row was planted, using seed obtained from Vilmorin of Paris. A first crop was cut on August 3, 1911, a second on September 11:—

	First crop.	Second crop.	Total.
	grammes.	grammes.	grammes.
1A	464	360	824
2A	458	205	663
3A	541	532	1073
4A	1349	774	2123

Each of the plots receives a dressing of 200 lbs. of ammonia salts per acre. Plot 1A has no other manure; 2A receives 3·5 cwt. superphosphate; 3A 200 lbs. sulphate of potash, 100 lbs. sulphate of soda and 100 lbs. sulphate of magnesia; 4A has a complete mineral manure, receiving both superphosphate and alkali salts.

It is obvious that growth is favoured by complete manuring. The smaller

difference between the plots at the second cutting was probably a result of the exceptionally dry condition of the season. The low yield on 2A is perhaps the consequence of the excessive removal of alkalis from the soil in previous years owing to the relative abundance of phosphate.

Lastly, to deal with the value of *Lotus corniculatus* as a forage plant: Mr. R. G. Stapledon, of the Royal Agricultural College, Cirencester, in an interesting report on the Flora of certain Cotswold pastures, published in the 'Scientific Bulletin' of the College (No. 2, 1910, pp. 29—46), makes the following statement in an appendix to his report:—

Stebler includes *Lotus corniculatus* amongst "the best forage plants." It is indigenous to all parts of England and thrives on all kinds of soil; it is common alike on moors, poor dry land at high elevations and on sandy maritime golf-links. It contributes very largely to the keep of sheep which graze and thrive excellently on the stunted pastures of the latter situations. The sward of the "burrows" by the River Torridge in Devon may be given as an example. . . . The Leguminosæ are represented by *Lotus corniculatus;* the variety *L. crassifolius* is also common.

Prof. T. H. Middleton, in his paper on "The Improvement of Poor Pastures,"* mentions *Lotus corniculatus* as responding well to basic slag, although, as he says, it is not such a good "soil improver" as *Trifolium repens.*

Lotus corniculatus is referred to in Sutton's 'Permanent and Temporary Pastures' as a very useful cropper; it is described as standing the severest drought and as able to grow on very light soils and even on clover-sick soils.

Lotus major, for some reason which is not apparent, is spoken of as inferior to *Lotus corniculatus.* Prof. Percival, in his 'Agricultural Botany,' refers to *Lotus corniculatus* seed as being usually adulterated with worthless *Lotus major* seed.

It will be of great importance not only to ascertain whether *Lotus corniculatus* is a valuable forage plant but to what extent, if at all, its value is to be correlated with the presence of the cyanophoric glucoside. Should it be ascertained that the glucoside is of special value, it will probably not be difficult to introduce into pastures the variety of the plant which is likely to be of maximum value. A step will also have been taken towards solving the problem to which we have called attention at the outset of this communication, as a method will have been devised which will be applicable to the study of other plants.

We have naturally turned our attention to other species of Lotus. We did not find hydrogen cyanide in *Lotus tetragonolobus* growing in the Saas valley in August last year nor in plants raised this year at Lewisham and Reading

* 'Journal of Agricultural Science,' vol. 1.

from seed supplied by Messrs. Vilmorin. Dr. Eyre has tested *Lotus siliquosus* growing in Bologna this year without finding cyanide. We have also failed in finding the cyanide in plants of *Lotus Bertholetti* (*peliorhynchus*), kindly placed at our disposal by Dr. Hugo Müller and by Mr. R. A. Robertson of St. Andrews University. But in *Lotus Jacobæus*, a native of the Canary Islands, we have found a plant which seems to be as rich both in cyanide and enzyme as *L. corniculatus*. We have raised this plant ourselves from seed. As it also contains acetone and the enzyme acts readily on linamarin, it is probable that the glucoside and enzyme are identical with those occurring in *Lotus corniculatus*. A plant was shown to Dr. Eyre in the Bologna botanic garden as *Lotus corniculatus* which he was informed was grown in Italy as a fodder plant; to judge from the specimen he has sent to us this was *Lotus Jacobæus*. We shall continue the study of this plant during the coming year and hope to be able to test the other species of Lotus not yet examined. It should be added that we have failed to find cyanide in *Hippocrepis comosa*, a plant which resembles *Lotus corniculatus* very closely.

We have to express our thanks for the assistance that has been given to us by Mr. Hall, Director of the Rothamsted Agricultural Experiment Station, by Dr. Keeble, Professor of Botany at the University College, Reading, as well as by many other willing helpers, particularly by Dr. J. V. Eyre, to whom we are so much indebted for the work he did for us abroad.

We desire also to ask botanists under whose notice this communication comes to favour us by carefully testing *Lotus corniculatus* for hydrogen cyanide, at the same time noting any botanical peculiarities the plant may show and the conditions under which it grows. We would ask them to inform us of the results they obtain and to favour us also with specimens whenever peculiarities are noticed. It will be best to cut the root well below ground and to send whole plants; we like to have several grammes of the leaf material, if possible, when testing for enzyme. We shall also be glad to have young plants or seed for further study.

In testing for cyanide we find it most convenient to make use of stout glass tubes, about $3\frac{1}{2}$ inches long and $\frac{1}{2}$ inch wide, provided with good corks. The leaf material having been pushed into the tube, two or three drops of chloroform or toluene are added and a slip of moist picrate paper is inserted; the tube is then corked up. It is conveniently incubated in a waistcoat breast-pocket or in the trousers pocket. When cyanide is present the paper reddens perceptibly within half an hour, as a rule; to make certain, the test should be prolonged over 24 hours. To prepare the picrate paper, slips of filter paper about $\frac{3}{8}$ inch wide are dipped into a solution of 5 grm.

picric acid and 50 grm. sodium carbonate in 1 litre of water; after draining the paper, it is hung from a pin to dry until it is only just perceptibly moist; it is then cut up into $\frac{3}{4}$-inch lengths and stored in a closed tube. It is well to keep a piece of the paper in each of the stock of tubes carried, so as to make sure that hydrogen cyanide has not been stored up in the cork.

City and Guilds College,
South Kensington, London.

Antelope Infected with Trypanosoma gambiense.

By Captain A. D. Fraser, R.A.M.C., and Dr. H. L. Duke.

(Communicated by Sir John Bradford, Sec. R.S. Received December 2, 1911,—Read January 25, 1912.)

The Sleeping Sickness Commission of the Royal Society, Uganda, 1908–10, showed that waterbuck, bushbuck and reedbuck could be readily infected with a human strain of *Trypanosoma gambiense,* and that clean laboratory-bred *Glossina palpalis* were capable of transmitting the virus from the infected antelope to susceptible animals.

In the present paper, observations which were made upon these antelope during the eight months subsequent to the Commission's departure from Uganda are recorded. Experiments are also described which show that the duiker—another species of antelope common in most parts of Uganda—can also be similarly infected with a human strain of *T. gambiense.* As regards the antelope employed by the Commission, six of the nine remained in apparently excellent health in April, 1911—roughly, a year after they were infected.

Until Bushbuck 2428 escaped from the kraal, and Bushbuck 2372 died 338 days after its infection as the result of an accident, they had also been healthy. A *post-mortem* examination was made immediately after death in the case of Bushbuck 2372, but no evidence of trypanosomiasis was found.

Reedbuck 2427 appeared to be perfectly healthy for 200 days after it had been infected. It then died suddenly. At the *post-mortem* examination performed immediately after death the prescapular glands were found to be the size of a hazel-nut. On section they were hæmorrhagic. There were numerous petechiæ on the mucous membrane of the mesentery. The